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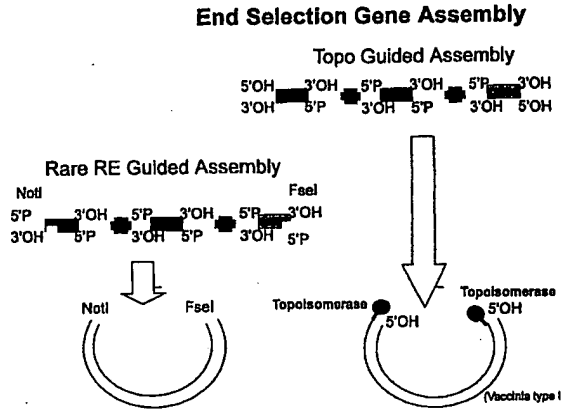
(54) Title: END SELECTION IN DIRECTED EVOLUTION



(57) Abstract

This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by the use of non–stochastic methods of directed evolution (DirectEvolution™). A particular advantage of end–selection–based methods is the ability to recover full–length polynucleotides from a library of progeny molecules generated by mutagenesis methods. These methods include non–stochastic polynucleotide site–saturation mutagenesis (Gene Site Saturation Mutagenesis™) and non–stochastic polynucleotide reassembly (GeneReassembly™). This invention provides methods of obtaining novel enzymes that have optmized physical and/or biological properties. Through use of the claimed methods, genetic vaccines, enzymes, small molecules, and other desirable molecules can be evolved towards desirable properties. For example, vaccine vectors can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.

CLAIMS

What is claimed is:

1. A method for producing and isolating a polypeptide having at least one desirable property comprised of the steps of:

(a) subjecting a starting or parental polynucleotide set to a mutagenesis process so as to produce a progeny polynucleotide set; and

(b) subjecting the progeny polynucleotide set to an end selection-based screening and enrichment process, so as to select for a desirable subset of the progeny polynucleotide set;

whereby the above steps can be performed iteratively and in any order and in combination,

whereby the end selection-based process creates ligation-compatible ends,

whereby the creation of ligation-compatible ends is optionally used to facilitate one or more intermolecular ligations, that are preferably directional ligations, within members of the progeny polynucleotide set so as to achieve assembly &/or reassembly mutagenesis,

whereby the creation of ligation-compatible ends serves to facilitate ligation of the progeny polynucleotide set into an expression vector system and expression cloning,

whereby the expression cloning of the progeny polynucleotide set serves to generate a polypeptide set,

whereby the generated polypeptide set can be subjected to an expression screening process, and

whereby expression screening of the progeny polypeptide set provides a means to identify a desirable species, e.g. a mutant polypeptide or alternatively a polypeptide fragment, that has a desirable property, such as a specific enzymatic activity.

2. A method according to claim 1, wherein the mutagenesis process of step (a) is comprised of a process, termed saturation mutagenesis, for generating, from a codon-containing parental polypeptide template, a progeny polypeptide set in which a full range of single amino acid substitutions is represented at each amino acid position, comprising the steps of:

(a) subjecting a working codon-containing template polynucleotide to polymerase-based amplification using a 32-fold degenerate oligonucleotide for each codon to mutagenized, where each of said 32-fold degenerate oligonucleotides is comprised of a first homologous sequence and a degenerate N,N,G/T triplet sequence, so as to generate a set of progeny polynucleotides; and

(b) subjecting said set of progeny polynucleotides to recombinant expression such that polypeptides encoded by the progeny polynucleotides are produced;

whereby the above steps can be performed iteratively and in any order and in combination,

whereby, said method provides a means for generating all 20 amino acid changes at each amino acid site along a parental polypeptide template, because the degeneracy of the N,N,G/T sequence includes codons for all 20 amino acids,

whereby said method also provides a means for generating all possible fragments of a parental polynucleotide template, which in turn can lead to recombinant expression of a set of encoded polypeptide fragments, and

whereby expression screening of the progeny polypeptide set provides a means to identify a desirable species, e.g. a mutant polypeptide or alternatively a polypeptide fragment, that has a desirable property, such as a specific enzymatic activity.

3. A method according to claim 2, where said 32-fold degenerate oligonucleotide is comprised of a first homologous sequence, a degenerate N,N,G/T triplet sequence, and a second homologous sequence.

4. A method according to claim 2, where said 32-fold degenerate oligonucleotide is comprised of a first homologous sequence, a plurality of degenerate N,N,G/T triplets sequences, and a second homologous sequence.

5. A method according to claim 2, that is further comprised of the step of:

(c) subjecting a working codon-containing template polynucleotide to polymerase-based amplification using a nondegenerate oligonucleotide for each codon to mutagenized,

whereby the use of each nondegenerate oligonucleotide provides a means to generate a single point mutation in a working polynucleotide, which in turn provides a means to recombinantly express an encoded polypeptide having a corresponding single amino acid change as long as the mutation at the polynucleotide level is not silent, and

whereby said method also provides a means for generating all possible fragments of a parental polynucleotide template, which in turn can lead to the recombinant expression of a set of encoded polypeptide fragments, and

whereby expression screening of the progeny polypeptide set provides a means to identify a desirable species, e.g. a mutant polypeptide or alternatively a polypeptide fragment, that has a desirable property, such as a specific enzymatic activity.